9-14-1999

# Excretion of 14C-Fumonisin B1, 14C-Hydrolyzed Fumonisin B1, and 14C-Fumonisin B1-Fructose in Rats

William R. Dantzer
*Iowa State University*

Joan Hopper
*Iowa State University*

Kathleen Mullin
*Iowa State University*, kmullin@iastate.edu

Suzanne Hendrich
*Iowa State University*, shendric@iastate.edu

Patricia A. Murphy
*Iowa State University*, pmurphy@iastate.edu

# Excretion of $^{14}$C-Fumonisin $B_1$, $^{14}$C-Hydrolyzed Fumonisin $B_1$, and $^{14}$C-Fumonisin $B_1$-Fructose in Rats

William R. Dantzer,[†,‡] Joan Hopper,[§] Kathy Mullin,[§] Suzanne Hendrich,[†] and Patricia A. Murphy*[,†]

Department of Food Science & Human Nutrition and Laboratory Animal Resources, Iowa State University, 2312 Food Sciences Building, Ames, Iowa 50011

$^{14}$C-Fumonisin $B_1$ ($FB_1$) was produced by *Fusarium proliferatum* M-5991 in modified Myro liquid medium and purified to >95% purity with a specific activity of 1.7 mCi/mmol. Nine male and nine female F344/N rats were each dosed by gavage with 0.69 $\mu$mol of $^{14}$C-$FB_1$, $^{14}$C-hydrolyzed $FB_1$, or $^{14}$C-$FB_1$-fructose/kg body weight. Urinary excretion of $^{14}$C-$FB_1$ and $^{14}$C-$FB_1$-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of $^{14}$C-hydrolyzed $HFB_1$ was significantly greater ($P > 0.05$) in female rats as compared with male rats (17.3% vs 12.8% of the total dose, respectively). There were no significant ($P > 0.05$) differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 h after dosing. Lesser amounts continued to be excreted up to 9.25 h after dosing. Although biliary excretion of the $^{14}$C-$FB_1$, $^{14}$C-hydrolyzed $FB_1$, and $^{14}$C-$FB_1$-fructose was similar, increased urinary excretion of the $^{14}$C-hydrolyzed $FB_1$ as compared to $^{14}$C-$FB_1$ and $^{14}$C-$FB_1$-fructose indicated a greater absorption of the hydrolyzed form.

**Keywords:** *$^{14}$C-Fumonisin $B_1$; hydrolyzed fumonisin $B_1$; fumonisin $B_1$-fructose; excretion*

## INTRODUCTION

The fumonisins (FBs) are a family of mycotoxins including $FB_1$, $FB_2$, $FB_3$, and $FB_4$ (Gelderblom et al., 1988; Cawood et al., 1991) with $FB_1$ predominant. The FBs are produced by the maize pathogens *Fusarium proliferatum* and *Fusarium moniliforme*. Fumonisin consumption causes equine leucoencephalomalacia (Kellerman et al., 1990) and porcine pulmonary edema (Osweiler et al., 1992; Colvin and Harrison, 1992). Fumonisins also cause embryopathogenicity in chickens (Javed et al., 1993), developmental toxicity in hamsters (Floss et al., 1994), and kidney toxicity and liver cancer in rats (Gelderblom et al., 1991; Voss et al., 1993). Sharma et al. (1997) demonstrated in vivo apoptosis in mouse liver and kidney after doses of 0.35−8.7 $\mu$mol of $FB_1$/kg body weight (bw) were given subcutaneously. The effects of FBs on humans are not known. But epidemiological studies show significant correlations between high levels of FBs in corn consumed by humans and esophageal cancer (Sydenham et al., 1990; Rheeder et al., 1992; Chu and Li, 1994). Fumonisin $B_1$ is listed as a Class 2B carcinogen, a probable human carcinogen (IARC, 1993).

The study of fumonisin metabolism has been facilitated by the production of $^{14}$C-$FB_1$, which permits rapid analysis of the biological disposition of this compound. Fumonisin $B_1$ has been radiolabeled using $^{14}$C-acetate or -methionine in cultures of *F. moniliforme* or *F. proliferatum* in liquid medium (Norred et al., 1993; Blackwell et al., 1994; Lebepe-Mazur, 1993). In fasted rats, Norred et al. (1993) detected 80% and 2.3% of $FB_1$ administered by gavage (1.4 $\mu$mol of $^{14}$C-$FB_1$/kg bw) in feces and urine, respectively. Liver, kidney, and blood retained a total of 0.6% of the dose 96 h after treatment. In fed rats at 24 h after a dose administered by gavage (10.4 $\mu$mol of $^{14}$C-$FB_1$/kg bw), Shephard et al. (1992) detected 100% and trace levels of $FB_1$ in feces and urine, respectively. Trace levels of $^{14}$C-$FB_1$ were detected in liver, kidney, and blood of these rats. From these two studies, $FB_1$ absorption may be greater in fasted rats than in fed rats. Biliary excretion of FB has been suggested by observations of fecal recovery of $^{14}$C-FB given by intravenous or intraperitoneal routes (Norred et al., 1993; Shephard et al., 1992). Shephard et al. (1994) recovered 67% of an intraperitoneal dose (10.4 $\mu$mol of $^{14}$C-$FB_1$/kg bw) after 24 h in bile whereas 0.2% of a 0.4-$\mu$mol dose administered by gavage was detected in bile of fed rats. Other forms of fumonisins such as hydrolyzed $FB_1$ have not been evaluated for biliary excretion. Hopmans et al. (1997) analyzed by HPLC the excretion of unlabeled $FB_1$ at three doses. Administration of 0.69, 6.93, and 69.3 $\mu$mol of $FB_1$/kg bw to fed rats resulted in 7.4, 1.2, and 0.5% of the dose excreted in urine, respectively, confirming previous findings with radiolabeled $FB_1$ but also suggesting dose differences in absorption.

$FB_1$ may undergo reactions in food systems that alter its chemical structure and its toxicity. Alkaline hydrolysis of FB-containing corn produced hydrolyzed $FB_1$ (HFB), which was found to promote diethylnitrosamine (DEN)-initiated hepatocarcinogenesis nearly as well as $FB_1$ when fed to rats (Hendrich et al., 1993). Urinary excretion of $HFB_1$ was 2-fold greater than $FB_1$ in rats (Hopmans et al., 1997), suggesting a significant role for increased bioavailability in $HFB_1$ toxicity as compared

* To whom correspondence should be addressed (phone: 515-294-1970, fax: 515-294-8181, e-mail: pmurphy@iastate.edu).
[†] Department of Food Science & Human Nutrition.
[‡] Current address: Department of Food Science, Ohio State University, Columbus, OH.
[§] Laboratory Animal Resources.

10.1021/jf981340v CCC: $18.00 
Published on Web 09/14/1999

with $FB_1$. A Maillard-like reaction between $FB_1$ and a reducing sugar such as fructose can occur during heating (Murphy et al., 1996). Feeding $FB_1$-fructose reaction products caused no development of altered hepatic foci in DEN-initiated rats, whereas an equimolar amount of $FB_1$ promoted hepatocarcinogenesis readily (Lu et al., 1997). The seeming lack of toxicity of $FB_1$-fructose product(s) could not be explained by lesser absorption in Hopmans et al. (1997) because such products were absorbed to a greater extent than $FB_1$ in rats, based on relative urinary excretion. A $FB_1$-glucose reaction product, *N*-(carboxymethyl)-$FB_1$ has recently been isolated (Howard et al., 1998), but its toxicity is unknown. The mechanism of formation and the nature of the first products in the Maillard reaction are not well-characterized even after over 50 years of study (Ge and Lee, 1997). But fumonisin-sugar products might form during food processing and could diminish or alter fumonisin toxicity.

This study was designed to determine, with $^{14}C$-$FB_1$, the extent of urinary excretion of the low doses of 0.69 $\mu$mol of $FB_1$, $HFB_1$, and $FB_1$-fructose/kg bw used by Hopmans et al. (1997). We also analyzed biliary circulation of these three forms of fumonisin most likely to be found in foods.

## MATERIALS AND METHODS

Reagents were from Fisher Scientific (St. Louis, MO) unless noted otherwise. Milli-Q water (Millipore-Waters, Bedford, MA) was used throughout. All animal procedures and protocols were approved by the Iowa State University Animal Care and Use Committee. The $FB_1$ produced from *Fusarium* cultures is a class 2B carcinogen and was handled accordingly. We treated $HFB_1$ similarly. We followed Iowa State University Environmental Health and Safety guidelines for the use of $^{14}C$.

The 500-mL liquid cultures of *F. proliferatum* M5991 were prepared as in Dantzer et al. (1996a). These cultures were inoculated with Myro liquid medium (LM), containing $MgSO_4$ at only 0.5 g/L and 1.00% corn hull extract (modified Myro LM). The inoculum culture was incubated for 4 days on a rotary shaker at 220 rpm at 25 °C. Aliquots of 0.5 mL of inoculum were transferred to three replicate rubber-stoppered 125-mL Erlenmeyer flasks containing 50 mL of modified Myro LM. Compressed air was cleaned by passage through five 2-L plastic bottles containing air, 2 N KOH, 2 N KOH, distilled $H_2O$, and 2 N $H_2SO_4$, followed by a moisture trap and a 0.2-$\mu$m in-line filter. The purified air was bubbled through the *F. proliferatum*-inoculated culture flasks at 0.5 mL/min while the flasks were shaken on a rotary shaker at 220 rpm and 23 °C for 30 days. Aliquots of 1 mL were removed from the cultures every 7 days and frozen at −20 °C until analyzed for $FB_1$.

Glassware was cleaned with Nochromix laboratory glass cleaner (GODAX Laboratories, Inc, Takoma Park, MD) for >15 min. An 0.5-mL aliquot of a 4-day-old *F. proliferatum* M 5991 LM culture was transferred to a rubber-stoppered 125-mL Erlenmeyer flask. Air exiting the culture was passed through two $CO_2$ traps containing 2 N KOH. The flask was incubated with shaking at 220 rpm and 23 °C for 10 days. A 100-$\mu$L aliquot of culture was removed to obtain baseline $FB_1$ concentration. The ethanol containing 56 mCi/mmol universally labeled (U)-$^{14}C$-acetate (American Radiolabeled Chemicals, Inc., St. Louis, MO; Lot No. 960214) was evaporated, and the residue was dissolved in Milli-Q water. Preliminary results indicated that ethanol decreased production of $FB_1$ and cell density of *F. proliferatum* culture. Four equal aliquots of 250 $\mu$Ci of U-$^{14}C$-acetate were transferred into the *F. proliferatum* culture after 12, 15, 18, and 21 days for a total of 1.00 mCi of U-$^{14}C$-acetate. The culture was agitated on a shaker at 220 rpm and harvested at 25 days. The KOH traps were replaced every 24 h and counted in ScintiVerse BD scintillation fluid for 4 min using a Packard liquid scintillation analyzer, model 1900TR (Packard Instruments Co., Downers Grove, IL).

$^{14}C$-Fumonisin $B_1$ purification was performed according to Dantzer et al. (1996b) with modifications. Briefly, the 50 mL of $^{14}C$-$FB_1$-containing LM was filtered through a Whatman no. 1 filter, followed by 0.8- and 0.45-$\mu$m MSi filters (4.5 cm, nylon, Micron Separations, Inc., Westboro, MA). The $^{14}C$-$FB_1$ was fractionated on XAD-16, $C_8$, DEAE-Sepharose, and $C_{18}$ columns. The XAD-16 column was 30 × 2 cm with 50 g of Amberlite XAD-16 (Sigma Chemical Co., St. Louis, MO). The XAD column was washed with 150 mL of Milli-Q water followed by 150 mL of 50% acetonitrile in water. All other steps were as reported previously. After elution of the FB fraction from the $C_{18}$ column, the sample was concentrated by rotary evaporation at 45 °C, freeze-dried, weighed, rehydrated in 50% acetonitrile/water, and quantified for $FB_1$. Three aliquots, each containing 1.39 $\mu$mol of $^{14}C$-$FB_1$ were taken to dryness by rotary evaporation at 45 °C and used to prepare $^{14}C$-$FB_1$, $^{14}C$-$HFB_1$, and $^{14}C$-$FB_1$-fructose doses. The $^{14}C$-$FB_1$ dose was rehydrated in 10 mL of Milli-Q water. The $^{14}C$-$HFB_1$ was prepared by rehydration of 1.39 $\mu$mol of $FB_1$ in 5 mL of Milli-Q water and hydrolysis in a marble-capped test tube with 1 mL of 2 N KOH for 2 h in a boiling water bath. The pH of the $HFB_1$ solution was adjusted to <3 with 12 N HCl, loaded onto a PrepSep P479-$C_{18}$ extraction column, and washed with 20 mL of Milli-Q water. The $HFB_1$ was eluted with 10 mL of methanol (Hopmans et al., 1997). The methanol was removed by rotary evaporation at 45 °C. The $^{14}C$-HFB was redissolved in 10 mL of Milli-Q water. The $^{14}C$-$FB_1$-fructose was prepared by rehydration of 1.39 $\mu$mol of $^{14}C$-$FB_1$ in 10 mL of 50 mM $K_2HPO_4$, pH 7.0, containing 100 mM D-fructose and heating at 80 °C for 48 h (Hopmans et al., 1997). Ten percent of unreacted $FB_1$ remained in the $FB_1$-fructose mixture. The volume of the $FB_1$-fructose was adjusted to 10 mL with Milli-Q water. All three $^{14}C$-FB forms were stored at −20 °C until use.

The concentrations of $^{14}C$-$FB_1$ and -$HFB_1$ were estimated by HPLC analysis of *o*-phthaldialdehyde derivative using a Turner fluorometer (Corning 7-60 primary filter and Wratten 2a secondary filter, 15 $\mu$L flow cell) (Dantzer et al., 1996b). The purity of $^{14}C$-$FB_1$ was determined by comparing the amounts as measured by HPLC with the freeze-dried mass of the purified $^{14}C$-$FB_1$. The $^{14}C$-$HFB_1$ was quantified in a similar manner by comparison with an $HFB_1$ standard curve. The $HFB_1$ standard was prepared according to Hopmans et al. (1997). The $HFB_1$ standard was shown to be >95% pure by FAB-mass spectrometry (Hopmans et al., 1997; Mirocha, personal communication). Residual $FB_1$ in the $^{14}C$-$FB_1$-fructose dose was analyzed by HPLC (Dantzer et al., 1996b).

Eighteen Fisher 344/NHsd rats, nine male and nine female, at 7−8 and 9−10 weeks of age (bw 135−160 g), respectively, were randomly assigned to one of the treatment groups for the excretion study. All rats were housed individually and given AIN-93M diet (Reeves et al., 1993) and water ad libitum for 1 wk under a 12-h light/dark cycle. At the end of a 12-h light cycle, groups of three males and three females were administered 0.69 $\mu$mol of $^{14}C$-$FB_1$, $^{14}C$-$HFB_1$, or $^{14}C$-$FB_1$-fructose/kg bw by gavage. The rats were housed individually in metabolic cages, and fecal and urine samples were collected at 12-h intervals. At 84 h, the rats were sacrificed by $CO_2$ asphyxiation; blood was drawn by heart puncture; and the hearts, livers, lungs, kidneys, and brains were removed for $^{14}C$ analysis.

For the biliary excretion study, nine female 15-week-old Sprague−Dawley rats (bw 240−270 g) were used. All rats were housed individually and given AIN-93M diet and tap water ad libitum for 1 week under 12-h light cycle. Rats were anesthetized with 5 mL/kg bw of a 25% urethane (Sigma) solution intraperitoneally, and their bile ducts were surgically cannulated with silicone rubber tubing (0.3 mm i.d. × 0.6 mm o.d.; BrainTree Scientific, Braintree, MA). After cannulation, the abdomen was surgically closed, and 3 rats/treatment were immediately gavaged with 0.69 $\mu$mol of either $^{14}C$-$FB_1$, -$HFB_1$, or -$FB_1$-fructose/kg bw. All rats were placed on their left side on a hot water heating pad set at medium heat. Bile from each rat was collected into 1.5-mL graduated microcentrifuge tubes every 30 min until rats died or were sacrificed 9.5 h after dosing. Livers, kidneys, stomach washes, stomachs, and the

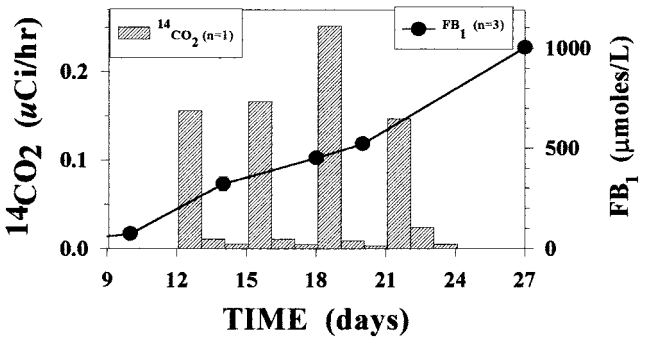


**Figure 1.** Production of $FB_1$ ($\mu$mol/L) and $^{14}CO_2$ ($\mu$Ci/h) by *Fusarium proliferatum* M-5991. Bars represent $^{14}CO_2$ production in 50 mL of modified Myro media spiked with 250 $\mu$Ci of U-$^{14}$C-acetate at days 12, 15, 18, and 21; $n = 1$. Line represents $FB_1$ production ($\mu$mol/L); $n = 3$.

first, second, and third sections (I, II, and III) of small intestines were collected after the rats died or at sacrifice, 9.5 h after dosing. All samples were stored at −20 °C until analysis.

Fecal samples, oven dried at 60 °C overnight, and intestinal tissues, frozen in liquid $N_2$, were ground in a porcelain mortar and pestle. Ground feces, intestinal tissues, stomach, stomach wash, kidney, liver, lung, brain, and blood were separately blended in a tissue homogenizer (model TR-10, Tekmar Co., Cincinnati, OH) at 60% power for 0.5−1 min in 5 mL of Milli-Q water, brought to a known volume of 10−35 mL with Milli-Q water, and quantified for $^{14}$C by scintillation analyzer. Quenching of tissues was measured with 0.5−1 mL of tissue extract with and without 18 400 dpm $^{14}$C-$FB_1$. Data were corrected using percent quenching in the respective sample.

A completely randomized design was used for statistical evaluation of urine and fecal excretion data in the 18 Fisher rats and for the biliary excretion data in the 9 Sprague Dawley rats. Differences among treatments were assessed by a Student's *t* statistic ($P \leq 0.05$) using SAS (version 6.03, 1995, Cary, NC).

## RESULTS AND DISCUSSION

*F. proliferatum* yielded 1200 $\mu$mol of $FB_1$/L between days 10 and 31 of culture (Figure 1). There was an average production of 0.18 $\mu$Ci of $^{14}CO_2$/h within the first 24-h period after each addition of 250 $\mu$Ci of $^{14}$C-acetate. The production of $^{14}CO_2$ decreased to undetectable levels 3 days after each addition of the $^{14}$C-acetate at an apparent logarithmic rate, suggesting that the production of acetate by the *F. proliferatum* culture was significantly greater than the addition of the labeled acetate. The culture was harvested at 24 days. The $FB_1$ was purified to >95% purity with a yield of 24 $\mu$mol and a specific activity of 1.7 mCi/mmol. $^{14}$C-Fumonisin $B_1$ measurement in biological samples allowed for improved monitoring of $FB_1$ as compared to HPLC fluorescence detection. But scintillation counting of $^{14}$C-$FB_1$ could not reveal any information on metabolic modification of $FB_1$ or its related forms.

The doses for the urine and fecal excretion study were 0.14 $\mu$mol of $^{14}$C-$FB_1$, $^{14}$C-$HFB_1$, or $^{14}$C-$FB_1$-fructose/mL with specific activities of 1.7, 1.2, and 1.8 mCi/mmol, respectively. The lower specific activity of the $HFB_1$ suggested that part of the U-$^{14}$C-acetate was incorporated into the tricarboxylic side chains of $FB_1$ that were removed during hydrolysis to produce $HFB_1$.

Quenching in the blood and liver fractions was determined to be 86 and 28%, respectively, for added $^{14}$C-$FB_1$. All other tissues had negligible quenching (data not shown). All data were corrected for quenching.

Recently, Howard et al. (1998) identified *N*-(carboxymethyl)-$FB_1$ as a reaction product of $FB_1$ and glucose. Yaylayan and Huyghues-Despointes (1994) reported that nonenzymatic browning reaction products from fructose are much more complicated than those from glucose due to the nature of the products formed from a ketose. In addition, our reaction time between $FB_1$ and fructose was 4-fold longer than that reported by Howard et al. (1998), which probably means that our $FB_1$-sugar adducts were a much more complex mixture, as is usually seen with the formation of Amadori products (Labuza, 1994). For the sake of simplicity in this paper, we named our fructose-$FB_1$ reaction product mixture, $FB_1$-fructose. Our limited attempts to identify the products produced in the $FB_1$-fructose (and -glucose) model systems by mass spectrometry have been unsuccessful to date. A number of *o*-phthalaldeyhyde (OPA) fluorescent peaks were detected in the FB-fructose reaction mixture, but we would not expect anything reacting with the FB amine group to be detected by amine derivatization.

Excretion of $^{14}$C in urine over time did not differ significantly between genders, except for $HFB_1$ during the first excretion interval [female > male (Table 1)], so data were combined ($n = 6$) (Figure 2). Fifteen percent, 4.4%, and 0.6% of the total $^{14}$C dose was excreted in urine for $HFB_1$, $FB_1$-fructose, and $FB_1$ respectively (Table 1). The average half-life for excretion of FB compounds was 10 h. There were only trace amounts of $^{14}$C-$FB_1$ excreted in urine with most of the urinary excretion occurring in the first 12 h (Figure 2). The urinary excretion of $HFB_1$ persisted for up to 60 h after dosing with maximum excretion occurring between 12 and 24 h. The urinary excretion of $^{14}$C-$FB_1$-fructose persisted for 24 h with the maximum excretion occurring in the first 12 h. The pattern of excretion of $HFB_1$ and of $FB_1$-fructose resembled one-compartment models of elimination. Amounts of 2-5-fold greater $^{14}$C-$HFB_1$ than $FB_1$ were excreted in urine, suggesting that $HFB_1$ was better absorbed than $FB_1$ in these rats. Dietary $HFB_1$ was nearly as toxic as $FB_1$ during the promotion phase of a two-stage model of rat hepatocarcinogenesis (Hendrich et al., 1993). Because the similarity between $HFB_1$ and $FB_1$ in toxicity may be accounted for by the greater bioavailability of $HFB_1$, $FB_1$ would seem to be more toxic at the cellular level than $HFB_1$. But in primary rat hepatocytes, HFBs were more cytotoxic than FBs as measured by lactate dehydrogenase leakage (Gelderblom et al., 1993). The toxic dose of FBs was 1 mM or more in the hepatocyte cultures. Such high concentrations would be highly unlikely to be achievable through dietary exposures, given the very limited apparent absorption of $FB_1$. The hepatocyte culture studies are probably not helpful in explaining $FB_1$ toxicity in vivo.

Total excretion of $^{14}$C-$FB_1$-fructose was 8-fold greater than $FB_1$, suggesting that $FB_1$-fructose may have greater bioavailability than $FB_1$ in these rats (Table 1). Fumonisin $B_1$-fructose product(s) seem to be detoxified forms of $FB_1$ during the promotion phase of a two-stage rat hepatocarcinogenesis model (Lu et al., 1997). The mechanism cannot be due to reduced absorption of FB-fructose. Blocking the FB amine group prevented toxicity in primary rat hepatocyte cultures as well as in vivo (Gelderblom et al., 1993). Perhaps the blocked amine group sterically prevents the interaction of FBs with their molecular sites of action.

**Table 1. Percent Recovery of $^{14}$C from 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, and $^{14}$C-FB$_1$-Fructose/kg Body Weight in Rats[a]**

| | percent dose | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | urine | | feces | | tissues | | recovery | |
| total dose | male | female | male | female | male | female | male | female |
| FB$_1$ | 0.4[a] | 0.7[a] | 85 | 95 | 0.28 | 2.72 | 86 | 99 |
| HFB$_1$ | 12.8[c] | 17.3[d] | 87 | 91 | 0.14 | 1.31 | 100 | 110 |
| FB$_1$-fructose | 4.2[b] | 4.6[b] | 86 | 97 | 0.19 | 0.42 | 91 | 102 |

[a] Different superscripts indicate that the means were significantly different at $\alpha = 0.05$; $n = 3$ per gender and treatment.

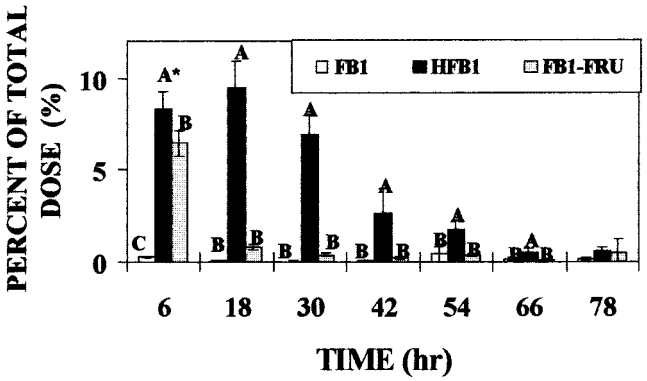


**Figure 2.** Urine excretion of $^{14}$C from 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, and $^{14}$C-FB$_1$-fructose/kg body weight by rats per 12-h interval. Error bars represent ± 1 standard deviation; $n = 6$. Bars within a time interval with different superscripts were different at $\alpha = 0.05$. FB$_1$-FRU = FB$_1$-fructose.

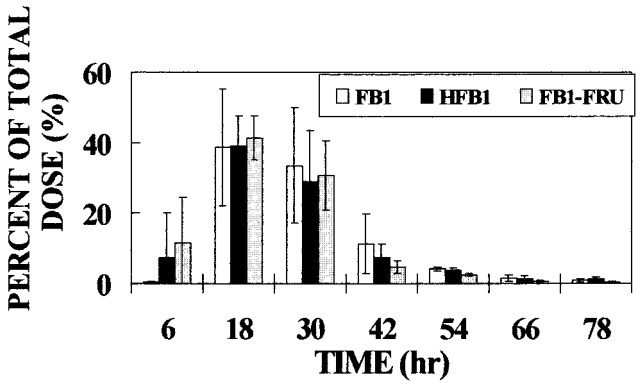


**Figure 3.** Fecal excretion of $^{14}$C from 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, and $^{14}$C-FB$_1$-fructose/kg body weight by rats per 12-h interval. Error bars represent ± 1 standard deviation; $n = 6$.

The total urinary excretion of 0.69 $\mu$mol of HFB$_1$ and FB$_1$-fructose as compared to FB$_1$ over 84 h was comparable to the previous study over 96 h at three dose levels (Hopmans et al., 1997). Both studies reported the same total percent urinary excretion of HFB$_1$ and relative absorptions of HFB$_1$ and FB$_1$-fructose as compared to FB$_1$. In the study by Hopmans et al. (1997), rats dosed with 0.69 $\mu$mol of FB$_1$-fructose or FB$_1$/kg bw excreted 4.2% of the total dose of FB$_1$-fructose and 7.4% of the total dose of FB$_1$ in the urine as compared with 4.4% and 0.6%, respectively, in our current study. Hopmans et al. (1997) correlated FB$_1$ and FB$_1$-fructose to the amount of OPA-HFB$_1$ in the hydrolyzed rat urine. Hydrolysis of urine produced a free amine group on the FB$_1$ molecule for OPA derivatization. In the current study, FB$_1$ and FB$_1$-fructose were measured as the amount of $^{14}$C detected in the rat urine. The differences in these two studies may reflect a difficulty in accurate estimation of very small quantities of FBs by sample extraction and HPLC analysis. Norred et al. (1993) found that 2−3% of a gavaged dose of 1.4 $\mu$mol of $^{14}$C-FB$_1$/kg bw was excreted in the urine of fasted Sprague−Dawley rats after 96 h, suggesting that their fasted rats had a greater absorption of FB$_1$ than our rats. Our dosing of rats occurred at the end of the light cycle. Rats do not eat much during the light cycle, so our rats should have been fairly comparable to rats fasted for 12 h. The apparent higher absorption by Norred's rats as compared to our rats could be attributed to differences in strain of rat or, more likely, to differences in absorption of FBs with the extent of food deprivation. In fed rats, food may make FBs less available for GI tract absorption.

The data for fecal excretion of $^{14}$C in male and female rats, dosed with the same compound, were combined because they were not significantly different according to gender (Figure 3). The pattern of fecal excretion of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, and $^{14}$C-FB$_1$-fructose followed a normal excretion of a compound through the fecal route (Casarett and Doull, 1991) with maximum excretion of $^{14}$C from the three FB forms between 12 and 24 h. After 60 h, only trace amounts of $^{14}$C were recovered in the feces from these rats. Total fecal excretion was not significantly different between FB compounds or gender and averaged 90% recovery of total dose after 84 h (Table 1).

**Table 2. Biliary Excretion of $^{14}$C from 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, and $^{14}$C-FB$_1$-Fructose/kg Body Weight in Rats over 4 h[a]**

| dose | percent excretion |
|---|---|
| FB$_1$ | 1.55 ± 2.51 |
| HFB$_1$ | 1.71 ± 1.99 |
| FB$_1$-fructose | 0.80 ± 0.82 |

[a] $n = 3$.

The total $^{14}$C recovered from the hearts, brains, livers, blood, kidneys, or lungs of rats dosed with 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, or $^{14}$C-FB$_1$-fructose/kg bw was not significantly different from zero for all rats, indicating that accumulation of these compounds did not occur after 84 h (data not shown).

In the biliary excretion study, Sprague−Dawley rats were used because the Fisher rats were not large enough for successful cannulation. The same FB treatments were used for the biliary excretion study as in the urinary excretion study. However, to obtain proper volumes, the 0.14 $\mu$mol of $^{14}$C-HFB$_1$ and $^{14}$C-FB$_1$-fructose/mL treatments were diluted with unlabeled 0.14 $\mu$mol of HFB$_1$ or FB$_1$-fructose/mL, which resulted in specific activities of 0.7 and 1.0 mCi/mmol, respectively. All nine of the cannulated female rats survived for 4 h after dosing. Biliary excretion of 0.69 $\mu$mol of $^{14}$C-FB$_1$, $^{14}$C-HFB$_1$, or $^{14}$C-FB$_1$-fructose/kg bw by female rats were not significantly different with an average of 1.35% and a range of 0.80 (FB$_1$ -fructose) to 1.17 (HFB$_1$) of the total $^{14}$C dose excreted 4 h after dosing (Table 2). Biliary excretion of the three FB compounds increased

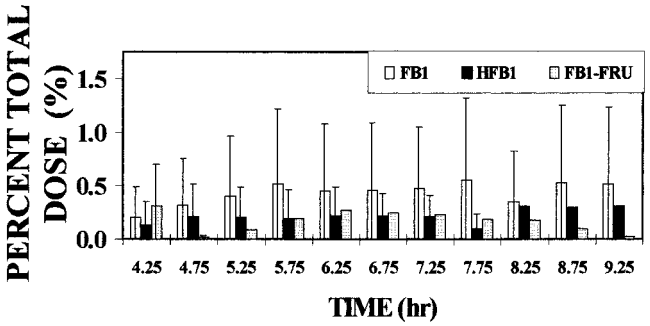


**Figure 4.** Biliary excretion of $^{14}C$ from 0.69 $\mu$mol of $^{14}C$-$FB_1$, $^{14}C$-$HFB_1$, and $^{14}C$-$FB_1$-fructose/kg body weight by female rats per 30-min interval. Error bars represent ± range; $n = 2$. Bars without error bars represent $n = 1$.

from 0 to 0.5% of the total dose per 0.5-h interval within 2 h after dosing. The three forms of FB continued to be excreted in the bile by the rats up to 9.5 h after dosing (Figure 4). There seemed to be no cyclical nature to $^{14}C$ excretion in the bile after administration of the dose by gavage. The large range seen in the biliary excretion data suggest the presence of fluorescent compounds that have been previously observed in rat bile (Hicks et al., 1984). Only two rats from the $^{14}C$-$FB_1$ dose and one rat from the $^{14}C$-$HFB_1$ and $^{14}C$-$FB_1$-fructose doses survived for 9.5 h. Shephard et al. (1994) recovered almost 7-fold less, 0.2% of their total $^{14}C$ dose in bile duct cannulated rats gavaged with $^{14}C$-$FB_1$ as compared to our rats. The rats used by Shephard et al. (1994) were not under anesthesia during gavage or bile collection. Half of the $^{14}C$ dose of the three forms of $FB_1$ were recovered in the stomach tissue and contents of our rats (50 ± 23% of the total dose), indicating that only about half of the dosed-FB compounds could have reached the small intestine of these rats (data not shown). Because these rats were under anesthesia throughout the duration of the experiment, there may have been a slower rate of absorption as compared with Shephard et al. (1994). The pooled intestines and bile samples from the rats contained 3 ± 6 and 3 ± 4% of the total dose, respectively. Kidneys and livers contained less than 1% of the $^{14}C$ dose. Total recovery of the $^{14}C$ doses from these rats of the bile excretion study averaged 63 ± 17%.

The 25-fold greater absorption of $^{14}C$-$HFB_1$ than that of $^{14}C$-$FB_1$ in male and female Fisher rats suggested that, once in circulation, $HFB_1$ was less toxic than $FB_1$, on a molar basis, because both have been shown to be equally toxic on a dietary basis to rats (Hendrich et al., 1993). Detoxification of $FB_1$ by the formation of $FB_1$-fructose was not the result of decreased absorption since $^{14}C$-$FB_1$-fructose was absorbed 8-fold more than $^{14}C$-$FB_1$ by these rats. These data complement and extend the findings of Hopmans et al. (1997), suggesting that $HFB_1$ or $FB_1$-fructose were absorbed more than $FB_1$. In addition, there were no differences in biliary excretion of $^{14}C$-$FB_1$, $^{14}C$-$HFB_1$, or $^{14}C$-$FB_1$-fructose in female Sprague–Dawley rats, lending additional support to the likelihood that the observed decrease in urinary excretion of $FB_1$ as compared with $HFB_1$ or $FB_1$-fructose was due to decreased absorption of $FB_1$ relative to $HFB_1$ or $FB_1$-fructose in rats.

## LITERATURE CITED


Blackwell, B. A.; Miller, J. D.; Savard, M. E. Production of carbon 14-labeled fumonisin in liquid culture. *J. AOAC Int.* **1994**, *77*, 506–511.

Cawood, M. E.; Gelderblom, W. C. A.; Vleggaar, R.; Behrend, Y.; Thiel, P. G.; Marasas, W. F. O. Isolation of the fumonisin mycotoxins: a quantitative approach. *J. Agric. Food Chem.* **1991**, *39*, 1958–1962.

Casarett, L. J.; Doull, J. *Toxicology, The Basic Science of Poisons*, 4th ed.; Amdur, M. O., Doull, J., Klaassen, C. D., Eds.; Pergamon Press: New York, 1991; pp 69–71.

Chu, F. S.; Li, G. Y. Simultaneous occurrence of fumonisin $B_1$ and other mycotoxins in moldy corn collected from the People's Republic of China in regions of high incidences of esophageal cancer. *Appl. Environ. Microbiol.* **1994**, *60* (3), 847–852

Colvin, B. M.; Harrison L. R. Fumonisin-induced pulmonary edema and hydrothorax in swine. *Mycopathologia* **1992**, *117*, 79–82.

Dantzer, W. R.; Pometto, A. L., III; Murphy, P. A. Fumonisin $B_1$ production by *Fusarium proliferatum* strain M5991 in a modified myro liquid medium. *Nat. Toxins* **1996a**, *4*, 168–173.

Dantzer, W. R.; Hopmans, E.; Clark, A.; Hauck, C.; Murphy, P. A. Purification of Fumonisin $B_1$ from liquid cultures of *Fusarium proliferatum*. *J. Agric. Food Chem.* **1996b**, *44*, 3730–3732.

Floss, J. L.; Casteel, S. W.; Johnson, G. C.; Rottinghaus, G. E.; Drause, G. F. Developmental toxicity in hamsters of an aqueous extract of *Fusarium moniliforme* culture material containing known quantities of fumonisin $B_1$. *Vet. Hum. Toxicol.* **1994**, *36*, 5–10.

Ge, S. J.; Lee, T. C. Kinetic significance of the Schiff base reversion in the early-stage Maillard reaction of a phenylalanine-glucose aqueous model system. *J. Agric. Food Chem.* **1997**, *45*, 1619–1623.

Gelderblom, W. C. A.; Jaskiewicz, K.; Marasas, W. F. O.; Thiel, P. G.; Horak, R. M.; Vleggaar, R.; Kriek, N. P. J. Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* **1988**, *54*, 1806–1962.

Gelderblom, W. C. A.; Kriek, N. P. J.; Marasas, W. F. O.; Thiel, P. G. Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin $B_1$, in rats. *Carcinogenesis* **1991**, *12*, 1247–1251.

Gelderblom, W. C. A.; Cawood, M. E.; Snyman, S. D.; Vleggaar, R.; Marsas, W. F. O. Structure–activity relationships of fumonisins in short-term carcinogenesis and cytotoxicity assays. *Food Chem. Toxicol.* **1993**, *31* (6), 407–414.

Hendrich, S.; Miller, D. A.; Wilson, T. M.; Murphy, P. A. Toxicity of fumonisins in nixtamalized fumonisin corn-based diets fed to rats: effect of nutritional status. *J. Agric. Food Chem.* **1993**, *41*, 1649–1654.

Hicks, V. A.; Gunning, D. B.; Olson, J. A. Metabolism, plasma transport and biliary excretion of radioactive vitamin A and its metabolites as a function of liver reserves of vitamin A in the rat (Retinol). *J. Nutr.* **1984**, *114*, 1327–1333.

Hopmans, E. C.; Hauck, C. C.; Hendrich, S.; Murphy, P. A. Excretion of fumonisin $B_1$, hydrolyzed fumonisin $B_1$, and the fumonisin $B_1$-fructose adduct in rats. *J. Agric. Food Chem.* **1997**, *45*, 2618–2625.

Howard, P. C.; Churchwell, M. I.; Couch, L. H.; Marques, M. M.; Doerge, D. R. Formation of *N*-(carboxymethyl)fumonisin $B_1$, following the reaction of fumonisin $B_1$ with reducing sugars. *J. Agric. Food Chem.* **1998**, *46*, 3546–3557.

International Agency for Research on Cancer (IARC). Toxins derived from *Fusarium moniliforme*: Fumonisins $B_1$ and $B_2$ and Fusarin C. *IARC Monogr. Elimin. Carcinog. Risk Hum.* **1993**, *No. 56*, 445–466.

Javed, T.; Richard, J. L.; Bennett, G. A.; Dombrink-Kurtzman, M. A.; Bunte, R. M.; Koelkebeck, D. W.; Cote, L. M.; Leeper, R. W.; Buck, W. B. Embryopathic and embryocidal effects of purified fumonisin $B_1$ or *Fusarium proliferatum* culture material extract on chicken embryos. *Mycopathologia* **1993**, *123*, 185–193.

Kellerman, T. S.; Marasas, W. F. O.; Thiel, P. G.; Gelderblom, W. C. A.; Cawood, M.; Coetzeer, J. A. W. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin $B_1$. *Onderstepoort J. Vet. Res.* **1990**, *57*, 269–275.

Labuza, T. P. Interpreting the complexity of the kinetics of the Maillard reaction. In *Maillard Reactions in Chemistry, Food and Health*; Labuza, T. P., et al., Eds.; Royal Chemical Society: London, 1994; pp 176−181.

Lebepe-Mazur, S. Production of fumonisins by *Fusarium proliferatum* M5991 in submerged liquid culture medium and the metabolism study of C-14 labeled fumonisins in rats. Ph.D. Dissertation, Iowa State University, Ames, IA, 1993.

Lu, Z.; Dantzer, W. R.; Hopmans, E. C.; Prisk, V.; Cunnick, J. E.; Murphy, P. A.; Hendrich, S. Reaction with fructose detoxifies fumonisin $B_1$ while stimulating liver-associated natural killer cell activity in rats. *J. Agric. Food Chem*. **1997**, *45*, 803−809.

Murphy, P. A.; Hendrich, S.; Hopmans, E. C.; Hauck, C. C.; Lu, Z.; Buseman, G.; Munkvold, G. Effect of processing on fumonisin content of corn. In *Fumonisins in Foods*; Jackson, L., et al., Eds.; Plenum Press: New York, 1996; pp 323−334.

Norred, W. P.; Plattner, R. D.; Chamberlain, W. J. Distribution and excretion of $^{14}C$ fumonisin $B_1$ in male Sprague−Dawley rats. *Nat. Toxins* **1993**, *1*, 341−346.

Osweiler, G. D.; Ross, J. R.; Wilson, T. M.; Nelson, P. E.; Witte, S. T.; Carson, T. L.; Rice, L. G.; Nelson, H. A. Characterization of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings. *Vet. Diagn. Invest.* **1992**, *4*, 53−59.

Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.

Rheeder, J. P.; Marasas, W. F. O.; Thiel, P. G.; Sydenham, E. W.; Shephard, G. S.; Schalkwijk, D. J. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathologia* **1992**, *82*, 353−357.

Sharma, R. P.; Dugyala, R. R.; Voss, K. A. Demonstration of in-situ apoptosis in mouse liver and kidney after short-term repeated exposure to fumonisin $B_1$. *J. Comp. Pathol.* **1997**, *117*, 371−381.

Shephard, G. S.; Thiel, P. G.; Sydenham, E. W.; Alberts, J. F.; Gelderblom, W. C. A. Fate of a single dose of the 14C-labeled mycotoxin, fumonisin $B_1$ in rats. *Toxicon* **1992**, *30* (7), 768−770

Shephard, G. S.; Thiel, P. G.; Sydenham, E. W.; Alberts, J. F. Biliary excretion of the mycotoxin fumonisin $B_1$ in rats. *Food Chem. Toxicol.* **1994**, *32* (5), 489−491.

Sydenham, E. W.; Thiel, P. G.; Marasas, W. F. O.; Shephard, G. S.; Van Schalkwyk, D. J.; Koch, K. R. Natural Occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence areas of the Transkei, Southern Africa. *J. Agric. Food Chem*. **1990**, *38*, 1900−1903.

Voss K. A.; Chamberlain W. J.; Bacon C. W.; Norred W. P. A preliminary investigation on renal and hepatic toxicity in rats fed purified fumonisin $B_1$. *Nat. Toxins* **1993**, *1*, 222−228.

Yaylayan, V. A.; Huyghues-Despointes, A. Chemistry of Amadori rearrangement products: synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 321−369.

Received for review December 10, 1998. Revised manuscript received May 24, 1999. Accepted July 26, 1999. This paper was supported in part by the Iowa Agriculture and Home Economics Experiment Station, Project 2406, a contributing project to North Central Regional Project, NC 129, and is published as Journal Paper No. 18189.

JF981340V